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AD NUMBER
AD835493
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TECHNICAL MANUSCRIPT 427

DISSEMINATED BLASTOMYCOSIS IN HAMSTERS
AFTER INTRAMUSCULAR, SUBCUTANEOUS,
AND INTRAPERITONEAL INOCULATION

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Francis X. Smith

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Special Operations Division
COMMODITY DEVELOPMENT AND ENGINEERING LABORATORY

and

Pathology Division
MEDICAL SCIENCES LABORATORY

Project 1C522301A059

April 1968

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ACKNOWLEDGMENTS

We acknowledge the kind assistance of Dr. Neeti Bohidar of the Biomathematics Division (CEIR, Inc.), who performed the statistical analysis of these data, and the technical assistance of Mr. William Annan.

ABSTRACT

Intramuscular or subcutaneous infections of either morphological phase of Blastomyces dermatitidis were fatal to hamsters. The intramuscular route was more often lethal to males than a subcutaneous infection. Female hamsters proved to be more resistant to the intramuscular injection.

Following spontaneous death or sacrifice, examination revealed an abscess at the site of injection. There were also regional and local lymph node involvement and extensive caseated nodules in the lungs.

The results indicate that (i) the $1 \times 10^5/0.1$ ml dose used in this study overcame the defense mechanisms of the host, and (ii) the hamster is an excellent laboratory animal for the study of B. dermatitidis.

I. INTRODUCTION

Blastomyces dermatitidis is a dimorphic fungus that causes four clinical types of disease in man: primary cutaneous, primary pulmonary, disseminated, and chronic cutaneous blastomycosis.^{1,2}

Because the cutaneous tissues are so frequently involved in this disease, it is interesting to note that parenteral infection has failed to cause systemic disease in mice,³ hamsters,⁴ or man.²

Recently, Denton et al.⁵ first successfully isolated B. dermatitidis from soil. In the present investigation, the virulence of this soil isolate was studied during a 63-day period after intramuscular, subcutaneous, and intraperitoneal inoculation of male hamsters. The relative ability to produce disseminated disease in male and female hamsters after an intramuscular infection was also investigated.

II. MATERIALS AND METHODS

A. ANIMALS

All animals used were male or female hamsters, Mesocricetus auratus, weighing 80 to 100 grams.

B. INOCULA

Peptone glucose agar plates⁶ were spread with a thick suspension of the yeast phase of B. dermatitidis (Denton isolate). The plates were incubated at room temperature for 8 days, during which a growth of whitish, dry aerial mycelium with many conidia was produced. Cultures were harvested by adding several ml of sterile physiological saline and gently scraping the growth into suspension with a wire loop. The suspensions were pooled and rendered homogenous by subjecting them to 10 complete cycle strokes in a tissue grinder. The percentages of conidia (90%) and hyphal elements (10%) were determined by hemocytometer count. A viable count was made on the peptone medium, and a suspension was prepared in sterile physiological saline to contain 1×10^5 viable particles per 0.1 ml (Fig. 1).

A yeast-phase inoculum was prepared by seeding peptone medium plates with a heavy suspension of the yeast phase of the Denton isolate. After the plates had been incubated at 37 C for 7 days, they were flooded with sterile physiological saline and the growth was gently scraped into suspension with a wire loop. The various suspensions were pooled, and clumps

were broken up by subjecting the material to 10 complete cycle strokes in a tissue grinder. A viable count was made, and the preparation was diluted to contain 1×10^5 yeast-phase cells per 0.1 ml (Fig. 2).

C. VIRULENCE OF B. DERMATITIDIS IN HAMSTERS

1. Mycelial-Phase Inoculum

Sixty male hamsters were divided into three groups of 20 animals. Group 1 received an intramuscular injection of 0.1 ml of the mycelial inoculum containing 1×10^5 viable particles; group 2 received the same dose subcutaneously; and group 3, intraperitoneally. Nineteen female hamsters were also injected intramuscularly with this dose. All intramuscular and subcutaneous injections were made in the thigh of the right leg.

Animals that died spontaneously were examined grossly. Impression smears in lactophenol cotton blue were made from parenteral (Fig. 3), lymph node, epididymal, testicular, and lung (Fig. 4) lesions. Exudate from the parenteral and lung lesions was cultured at room temperature on peptone agar containing 0.05 mg chloramphenicol/ml and 0.5 mg Actidione/ml. Sixty-three days after infection, the surviving hamsters were sacrificed by an intraperitoneal injection of pentobarbital. After a gross examination, impression smears were made from any visible lesion, and exudate was obtained from parenteral abscesses and lung lesions for culture at room temperature. The isolated fungi resembled B. dermatitidis by microscopic examination; this was confirmed by conversion of randomly selected isolates to the yeast phase.

2. Yeast-Phase Inoculum

Male hamsters were divided into groups of 16 and 13 animals and injected intramuscularly and subcutaneously, respectively, with 0.1 ml containing 1×10^5 yeast-phase cells. The procedure following spontaneous death and sacrifice at 63 days was the same as outlined above for the mycelial inoculum.

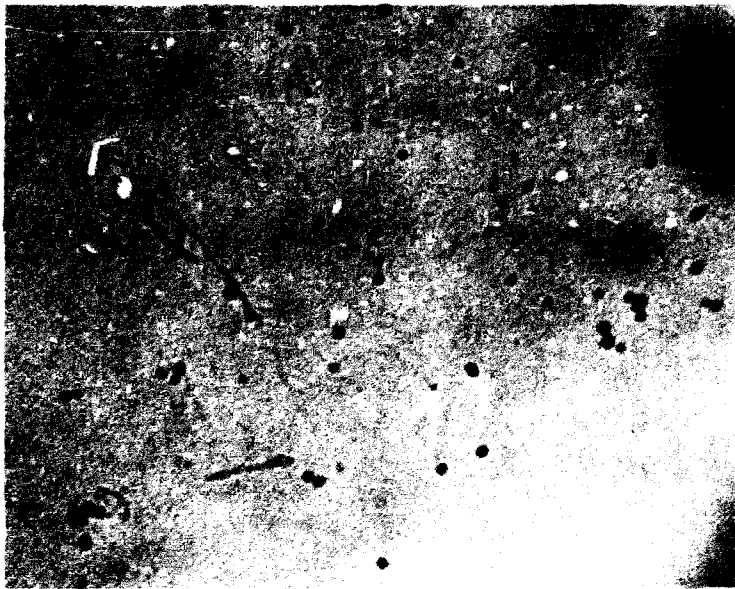


Figure 1. Mycelial-Phase Inoculum.

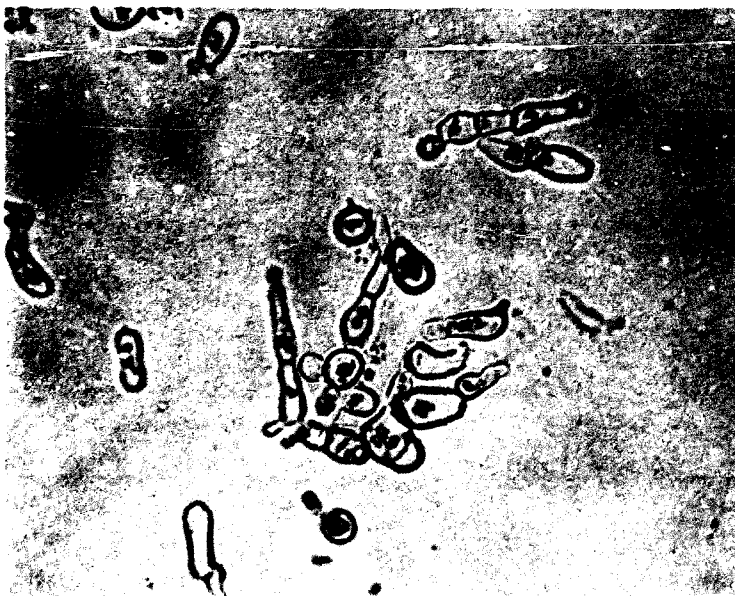


Figure 2. Yeast-Phase Inoculum.

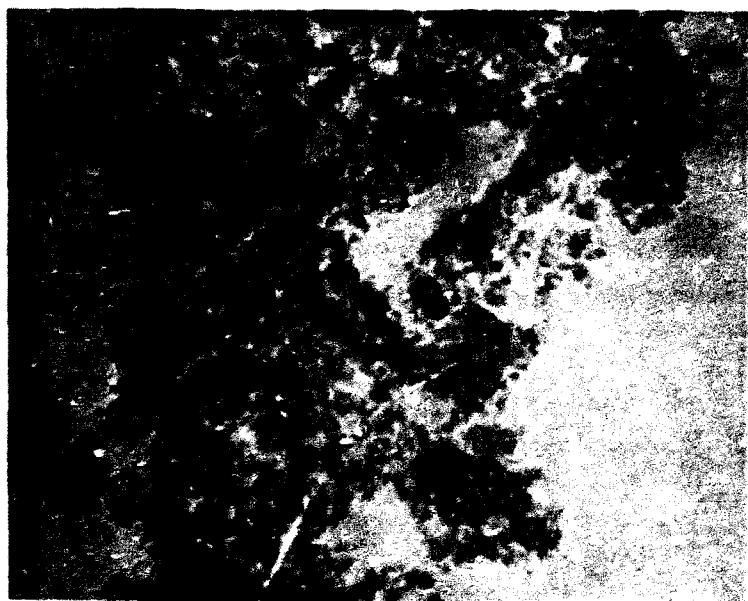


Figure 3. Impression Smear from the Site of Infection (right leg).

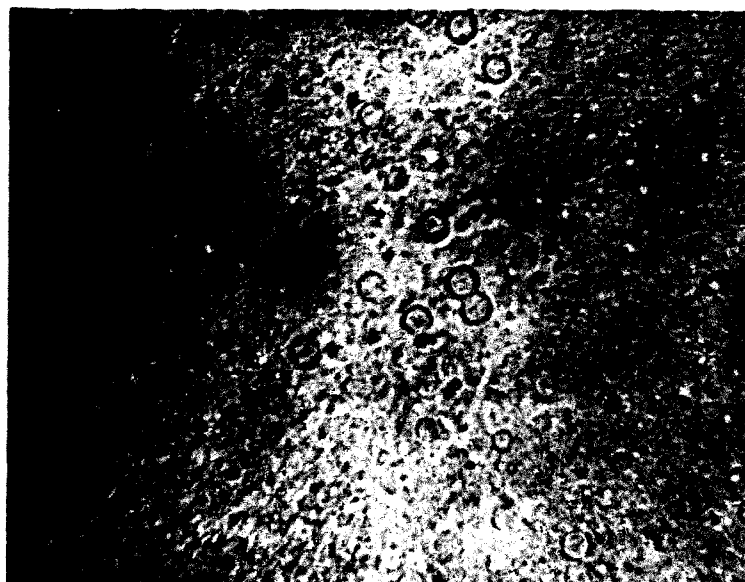


Figure 4. Impression Smear from the Lung of a Hamster Injected Intramuscularly.

III. RESULTS

Hamsters that died with 63 days after intramuscular or subcutaneous inoculation were graded 4 (Table 1). A gross examination of these animals revealed pulmonary lesions (Fig. 5) that varied from scattered small nodules to larger caseous nodules and abscesses. Extensive pleural adhesions were almost always present. In these animals, the regional and local lymph nodes were frequently involved (Fig. 6); they were large, hard, white, and caseous. An abscess, which varied in size from 0.1 to 1.5 cm, was present in the right leg at the site of injection. A small number of these abscesses were open and emitted a purulent fluid. Occasionally, multiple small abscesses were observed at the site of injection (Fig. 7).

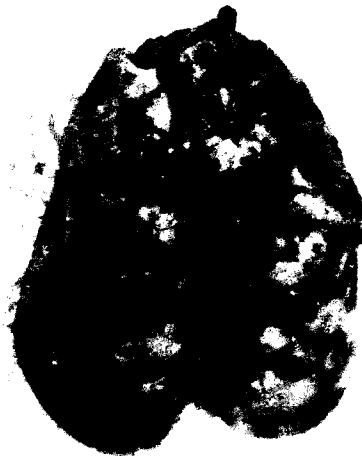


Figure 5. Pulmonary Lesions in the Lungs of an Intramuscularly Injected Hamster.



Figure 6. Right Leg, Lymphatic and Pulmonary Involvement in Intramuscularly Injected Hamster.



Figure 7. Multiple Small Abscesses in Right Leg of Intramuscularly Injected Hamster.

Animals that survived the 63-day period of the experiment but exhibited lung involvement were graded 3. These hamsters exhibited the typical abscess at the site of injection, and the regional and local lymph nodes were often involved. Only a few pinpoint white abscesses were present in the lungs. The lesions involved from one to several lobes, and an occasional pleural adhesion was observed.

Those hamsters that had regional and local lymph node involvement and an abscess at the site of injection but no lung disease were graded 2.

Hamsters that were injected parenterally and exhibited a typical lesion at the site of injection but showed no other evidence of disease were graded 1.

An abscess at the site of injection was observed in 16 female hamsters. The lesions did not appear to be as extensive, however, as those observed in comparably infected males. Three females exhibited no abscesses at the site of injection, but one of these animals died of mycotic pneumonia and a second exhibited regional and local lymph node involvement.

The hamsters injected intraperitoneally that died during the course of the experiment were also graded 4. In all of these animals, extensive caseated nodules and abscesses were scattered throughout both lungs, and pleural adhesions were present.

Most of the animals in this group exhibited lesions in the testicles and epididymis. Mesenteric lymph node involvement and splenic lesions were less frequently observed. Necropsy of the one surviving animal revealed B. dermatitidis lesions in the spleen and epididymis.

TABLE 1. CLINICAL CONDITION OF HAMSTERS AT 63 DAYS

Category ^{a/}	Number of Males						Number of Females
	Mycelial			Yeast		Mycelial	IM
	IM	SC	IP	IM	SC		
4	19	9	19	16	4		12
3	0	4	0	0	3		1
2	1	2	0	0	4		4
1	0	5	1	0	2		1
0	0	0	0	0	0		1
Total	20	20	20	16	13		19

- a. 4 = Animal died, lungs totally involved.
 3 = Lung disease present, but animal viable.
 2 = Lungs free of disease, but lymph node involvement.
 1 = Abscess observed only at site of injection.
 0 = No evidence of disease.

A statistical analysis (normal approximation to binomial distribution test) of the percentage of animals in each group that died spontaneously is summarized in Table 2. The figures obtained revealed that there is no significant difference in lethal effect among the following pairs: intraperitoneal male (mycelial) vs. intramuscular male (mycelial), intramuscular male (mycelial) vs. intramuscular male (yeast), and subcutaneous male (mycelial) vs. subcutaneous male (yeast).

TABLE 2. STATISTICAL COMPARISON OF EFFECTS OF ROUTES OF INFECTION, SEX, AND MORPHOLOGICAL PHASES

Comparisons		Animals that Died, %		Values of U	Significance ^{a/}
IP male mycelial	vs. IM male mycelial	95	vs. 95	0.00	0
IP male mycelial	vs. SC male mycelial	95	vs. 45	4.98	**
IM male mycelial	vs. SC male mycelial	95	vs. 45	4.98	**
IM male mycelial	vs. IM female mycelial	95	vs. 63.11	2.938	**
IM male mycelial	vs. IM male yeast	95	vs. 100	0.866	0
SC male yeast	vs. IM male yeast	30.8	vs. 100	5.10	**
SC male mycelial	vs. SC male yeast	45.0	vs. 30.8	0.650	0

a. 0 = Not significant.

** = 1% level of significance.

This analysis revealed that either an intraperitoneal or an intramuscular mycelial injection was more often fatal to male hamsters than a subcutaneous mycelial injection. It also showed that an intramuscular yeast-phase injection was more often fatal to male hamsters than a yeast-phase injection by the subcutaneous route. A significantly larger number of male hamsters died from an intramuscular mycelial injection than did females.

In Table 3, only the animals that died spontaneously were considered (79 of 108). The coefficient of variation, similar for all groups, ranged from a low of 20% in the intramuscular mycelial male group to a high of 25.7% in the intramuscular mycelial female animals.

TABLE 3. STATISTICAL ANALYSIS OF ANIMALS THAT DIED

Growth Phase and Route of Injection	No. Dead	Mean Time to Death, days	Standard Deviation	Coefficient of Variation, %
Mycelial phase IM male	19	39.2	7.81	20
Mycelial phase SC male	9	48.4	10.97	22
Mycelial phase IM female	12	42.9	11.04	25.7
Yeast phase IM male	16	47.2	10.25	21.7
Yeast phase SC male	4	52.8	12.28	23
Mycelial phase IP male	19	30.9	6.53	21

IV. DISCUSSION

The exact manner in which B. dermatitidis enters the human body in the natural disease is not known,² but there has been evidence of pulmonary involvement in so high a percentage of cases that it is believed that the disease results from inhalation of spores. In this study, however, intramuscular or subcutaneous injection into hamsters of either morphological form of this isolate of B. dermatitidis not only resulted in a local lesion, but often in dissemination to the lungs and death from mycotic pneumonia. Wilson and Plunkett³ theorized that the ability to resist B. dermatitidis in man is because of immunological mechanisms. It appears that the 1×10^5 /0.1 ml dose used in this study overcame the defense mechanisms of the hamster and permitted development of a pulmonary disease after parenteral injection. Because various species of animals differ in their susceptibility to B. dermatitidis,⁷ it is possible that individuals within a species would similarly differ. It is interesting to theorize on the sequence of events that would follow if an immunologically deficient human being were injected subcutaneously or intramuscularly with an optimal dose of B. dermatitidis organisms.

Salfelder⁴ failed to observe dissemination in hamsters after a subcutaneous injection of 0.1×10^6 yeast-phase cells of another B. dermatitidis isolate. Apparently different strains of B. dermatitidis differ in their virulence for the hamster, and virulence is related in some way to the ability of the isolate to diffuse through the tissues. Possibly the high virulence of this soil isolate facilitated its initial isolation in the mouse.

Because men contract the disease nine times more often than women,⁸ it is interesting to note (Table 1) that male hamsters are more susceptible to intramuscular infection than female hamsters. Because blastomycosis is believed to be acquired from an exogenous source, men would more readily come into contact with this fungus than women. This study does suggest, however, that sex of the individual, possibly along with the frequency of contact with the organism, does play a role in the morbidity of this disease.

Although 95% of the male hamsters died after intramuscular inoculation and only 63% of the females did, Table 3 shows that the mean time to death for both groups was similar. Thus, it appears that male hamsters are uniformly susceptible to intramuscular inoculation of B. dermatitidis, while females, as a group, are more resistant to the disease. Susceptible females within this group, however, exhibit a mean time to death comparable to that of males.

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Security Classification

17

DOCUMENT CONTROL DATA - R & D		
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)		
1. ORIGINATING ACTIVITY (Corporate author)		2a. REPORT SECURITY CLASSIFICATION
Department of the Army Fort Detrick, Frederick, Maryland, 21701		Unclassified
		2b. GROUP
3. REPORT TITLE		
DISSEMINATED BLASTOMYCOSIS IN HAMSTERS AFTER INTRAMUSCULAR, SUBCUTANEOUS, AND INTRAPERITONEAL INOCULATION		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)		
5. AUTHOR(S) (First name, middle initial, last name)		
Marshall E. Landay Francis X. Smith Edwin P. Lowe John Q. Mitten		
6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
April 1968	17	8
8a. CONTRACT OR GRANT NO.	8b. ORIGINATOR'S REPORT NUMBER(S)	
8. PROJECT NO. 1C522301A059	Technical Manuscript 427	
9.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
4.		
10. DISTRIBUTION STATEMENT		
Qualified requesters may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized.		
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY
		Department of the Army Fort Detrick, Frederick, Maryland, 21701
13. ABSTRACT		
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14. Key Words		
* <u>Blastomyces dermatitidis</u> *Hamsters Inoculation route *Fungi		

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